A RAMAN INVESTIGATION INTO THE SELF-ASSOCIATION OF 5′-GMP IN NEUTRAL AQUEOUS SOLUTION

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1. Introduction

It is a well-known fact that on cooling solutions of 5′-GMP at pH 5 under the correct conditions, one obtains a clear viscous gel [1,2]. Furthermore, changes in the UV-absorbance and optical rotation of the solution occur during the gel formation. The ordered structure of 5′-GMP in acidic aqueous solution was interpreted in terms of a continuous helix with each nucleotide joined to the next by two hydrogen bonds [1]. This helix is stabilized by hydrogen bonding and stacking interactions [3].

In acidic aqueous solution, 3′-GMP also forms an ordered structure. This structure however is different from that formed by 5′-GMP at pH 5. From their X-ray data Gellert et al. [1] determined that 3′-GMP forms square planar tetramers stacked upon each other with the ribose and phosphate ends of the molecule protruding into the solvent.

Small and Peticolas [4] found that the differences between these two structures correspond to differences in the Raman spectra of the two isomers. They noted the appearance of a number of new bands in the Raman spectrum of the 5′-GMP gel at 0°C. Furthermore, they observed that the Raman-hypochromism of the line at 1487 cm^{-1} is essentially greater in 5′-GMP (pH 5), than in the 3′ isomer.

The structure of 5′-GMP in neutral aqueous solution has already been investigated by calorimetry [5] and IR-spectroscopy [6]. From their IR-measurements Miles et al. concluded that the structures of 5′-GMP at neutral pH and at pH 5 are different.

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2. Experimental

2.1. *Materials*

5′-GMP and 3′-GMP were purchased as disodium salts from Boehringer Mannheim (G.F.R.) and used without further purification. The GMP-solutions were obtained by dissolving the required amounts in de-ionized water or in D_2O. The desired pH values were adjusted by dropwise addition of HCl. NaCl was used to maintain a Na^+ concentration of 0.5 M in the neutral, and of 0.1 M in the acidic solutions. For intensity measurements, the GMP-solutions were prepared in 0.1 M sodium acetate buffer.

2.2. *Measurements*

Our Raman apparatus and our measuring technique have been described previously [7,8]. The 4880 Å-exciting line of a CR-8 argon ion laser yielded about 1.5 W in the sample. To demonstrate the temperature dependence of the intensity of the 1487 cm^{-1} band, spectra were recorded in the temperature interval 0–40°C in steps of two degrees. The peak heights of this line were measured and normalized by comparing them to their height at 0°C. The acetate band at 928 cm^{-1} was used as the reference line [8].

3. Results and discussion

Raman spectra of neutral H_2O and D_2O solutions of 5′-GMP at 0°C and 40°C are shown in figs.1 and 2. It can readily be noted that the intensities of the lines at 1322, 1367, 1487 and 1578 cm^{-1} in H_2O and at 1324, 1356, 1481 and 1580 cm^{-1} in D_2O

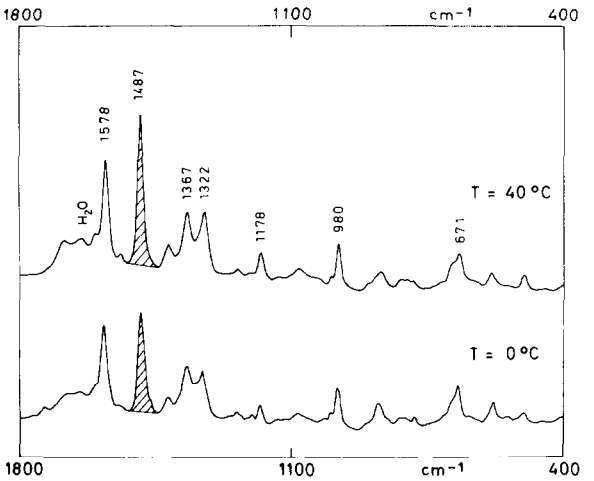

Fig.1. Raman spectra of 0.2 M 5′-GMP in H_2O at 0°C and 40°C, pH 7.5, C_{Na^+} = 0.5 M.

have markedly decreased in the spectra at 0°C. These vibrations have been attributed to guanine ring stretching modes [4,9]. This decrease in intensity of certain ring vibrations is probably due to structure formation and has been called 'Ramanhypochromism' by Small and Peticolas [10].

In fig.2, one can see that the line at 1670 cm^{-1} in the spectrum at 40°C, which is assigned to the stretching mode of the water solvated C = 0 group of

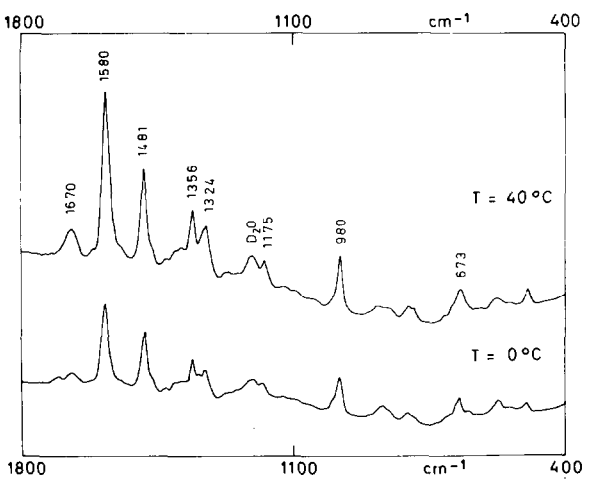

Fig.2. Raman spectra of 0.2 M 5′-GMP in D_2O at 0°C and 40°C, pD 7.5, C_{Na^+} = 0.5 M.

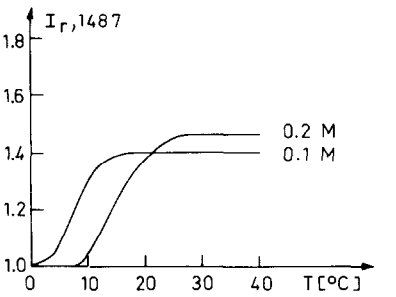

Fig.3. Plot of the intensity of the band at 1487 cm^{-1} in 0.1 M and 0.2 M 5′-GMP at pH 7.5 vs. temperature (ratio to intensity at 0°C).

guanine [11], is replaced by two different C = 0 frequencies (1670 and 1703 cm^{-1}) in the spectrum at 0°C. We assume that the appearance of the new line at 1703 cm^{-1} is caused by hydrogen bonding between the guanine bases. The fact that the band at 1670 cm^{-1} is still present at 0°C indicates that there is a certain amount of water hydrogen bonded C = 0 groups at that temperature. This may be due to the fact that the structure formation is incomplete.

In H_2O solution the line at 1487 cm^{-1} shows the strongest Ramanhypochromism on an absolute scale. In fig.3, the relative intensity of this line is plotted against temperature at two different 5′-GMP concentrations. The plots correspond to typical cooperative transitions with half conversion temperatures of about 8°C and 17°C and are almost identical to those obtained by calorimetry [5]. The Ramanhypochromism of this band may be due to hydrogen bonding at the N-7 position of guanine [13] and to stacking interactions.

Raman spectra of 5′-GMP and 3′-GMP in acidic H_2O solution have already been published [4]. In fig.4 spectra of the ordered forms of 5′-GMP at pH 5 and pH 7.5 and of 3′-GMP at pH 5 are presented. A comparison of these spectra shows that the line at 814 cm^{-1} in the spectrum of 5′-GMP (pH 5) cannot be detected in the spectra of 5′-GMP (pH 7.5) and 3′-GMP (pH 5). This line is thought to be due to the (H) - O - P - O - (H) symmetric stretching vibration of the dihydroxy species [12] and is characteristic for helix formation of the acidic form of 5′-GMP, for it disappears when the temperature is raised above the melting point [4]. There are two more major differences

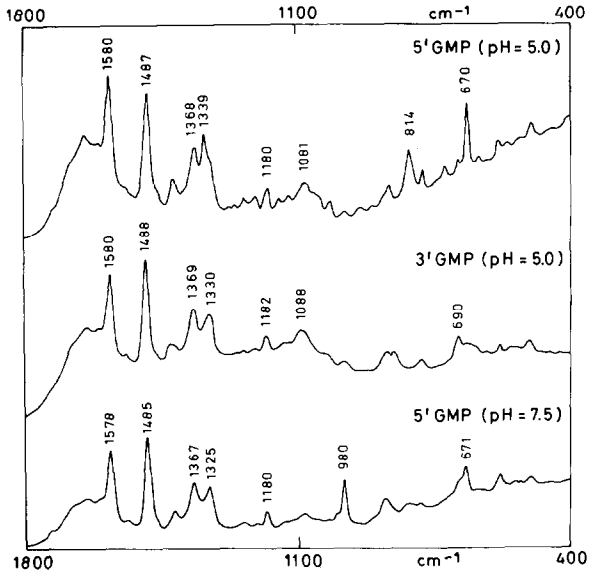

Fig.4. Raman spectra of 0.1 M 5'-GMP (pH 7.5, C_{Na^+} = 0.5 M), 0.1 M 3'-GMP (pH 5.0, C_{Na^+} = 0.1 M) and 0.1 M 5'-GMP (pH 5.0, C_{Na^+} = 0.1 M) in H_2O at 0°C.

between the spectrum of 5'-GMP at pH 5 and those of 3'-GMP (pH 5), and 5'-GMP (pH 7.5). Firstly, there is a very intense line at 670 cm^{-1} in the spectrum of 5'-GMP which is assigned to a ring stretching vibration [4,9]. If the ordered secondary structure is destroyed on heating, the shape and intensity of this band very closely resembles the same line in the spectrum of 5'-GMP (pH 7.5) at low and high temperature [4] (fig.1). Secondly, the line at 1325 cm^{-1} in the spectrum of 5'-GMP (pH 7.5), and at 1330 cm^{-1} in the spectrum of 3'-GMP (pH 5), which also corresponds to a ring vibration, shifted to 1339 cm^{-1} and increased in intensity in the spectrum of 5'-GMP at acidic pH. Shift and intensity increase of this line disappear if the temperature is raised above the melting point [4]. Furthermore, there are many rather weak lines in the spectrum of the 5'-GMP gel that cannot be detected in the spectra of the other forms. These lines disappear in the high temperature spectrum of 5'-GMP (pH 5) [4]. All these differences discussed so far have an obvious correspondence to structure formation of the 5'-GMP gel.

There are also differences between the spectra of 3'-GMP (pH 5) and 5'-GMP (pH 7.5) but they do not correspond to structure formation. The very intense line at 980 cm^{-1} in the 5'-GMP (pH 7.5) spectrum is confidently assigned to the $ROPO_3^{2-}$ stretching mode [11]. The band at 1088 cm^{-1} in the spectrum of 3'-GMP gel, assigned to the O-P-O-symmetric stretching vibration is only weak in the spectrum of 5'-GMP at neutral pH. Moreover, the ring stretching mode at 670 cm^{-1} in the 5'-GMP (pH 7.5) spectrum shifted to 690 cm^{-1} in the spectrum of the 3'-GMP gel.

None of these bands is affected by changes in the secondary structure [4] (fig.1). We assume that these differences are due to the different positions of the phosphate group in the two isomers and to the change of the pH value.

Figure 5 is a plot of the relative intensities of the 1487 cm^{-1} line vs. temperature for all three systems of this investigation. The concentrations for this plot were chosen with the purpose that the thermal transitions were in the same temperature range. One can see that the melting processes of 5'-GMP (pH 7.5), and 3'-GMP (pH 5), are somewhat more cooperative than those of 5'-GMP (pH 5). The strongest Raman-hypochromism occurs in the case of the 5'-GMP gel and the weakest in 5'-GMP at neutral pH. From this we can assume that the structure formation in the

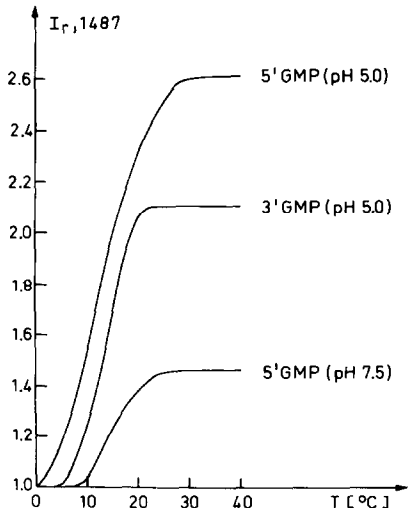

Fig.5. Plot of the intensity of the band at 1487 cm^{-1} in 0.2 M 5'-GMP (pH 7.5), 0.3 M 3'-GMP (pH 5.0) and 0.03 M 5'-GMP (pH 5.0) vs. temperature (ratio to intensity at 0°C).

acidic form of 5′-GMP is much more pronounced than in the neutral form.

The differences in the spectra of the ordered forms of 5′-GMP (pH 5) and 5′-GMP (pH 7.5), suggest that the secondary structures are different too. With the exception of the discussed differences which are due to the 5′ or 3′ position of the phosphate group, the spectra of the 3′-GMP gel and of the neutral form of 5′-GMP are very similar. From this we conclude that there is a great similarity in the structures of the ordered forms of these two monomers. The splitting of the 1670 cm^{-1} band and the Ramanhypochromism of the 1487 cm^{-1} line indicate that both the carbonyl group and the N-7 position are involved in hydrogen bonding in the ordered structure of 5′-GMP at neutral pH. We therefore favour a model of square planar tetramers for this structure similar to that suggested by Gellert et al. [1] for the 3′-GMP gel. This agrees very well with the IR measurements of Miles et al. [6]. However the Ramanhypochromism of the 3′-GMP gel is much stronger than that of the ordered form of 5′-GMP in neutral aqueous solution. Obviously the formation of the secondary structure is more complete in the 3′-GMP tetramers than in the neutral form of 5′-GMP.

Acknowledgement

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